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Problems in Treating Experimentally Induced Acute Hepatic Failure by Hemoperfusion or Cross Circulation

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Acute hepatic failure was induced in rats by galactosamine injection intraperitoneally (1 gm per kg). Twenty-four hours later rats were treated by hemoperfusion (HP) over encapsulated sorbents: cellulose acetate-coated charcoal, polyelectrolyte-coated XAD_4, a combination of both, or cross circulation with a healthy donor. Compared with control treatment (prevention of hypoglycemia by glucose infusion), the survival rate was not improved by HP or cross circulation: controls 19% vs. treated animals 0 to 17%. Extension of duration or increased frequency of HP gave the same survival rates. Computer simulation based on zero-order introduction of a possible toxin into a two-compartment model shows that HP up to 5 hr per day is not able to clear the body effectively from the assumed toxin if its partition coefficient exceeds a value of 50.

It is generally assumed that hepatic encephalopathy (HE) can be caused by a detrimental negative effect of a changed plasma composition on normal brain function (1).

Although progress in the study of objective quantification of brain malfunction certainly will improve our understanding of the pathogenesis of HE in the near future (2), the clinician cannot wait and watch the patient in the mean time. Many different techniques of clearing the blood of possible toxic substances and waste metabolites have been applied, varying from exchange transfusion to hemoperfusion (HP) (3).

In 1972, Chang (4) reported recovery of consciousness in Grade IV hepatic coma in man after charcoal HP treatment. Extension of this treatment to 56 patients showed no conclusive evidence of its beneficial effect partly due to the lack of controlled trials (5).

In 1981, Chang (6) published survival rates of fulminant hepatic failure in rats with severe galactosamine (Gal-N) hepatitis. During Grade II hepatic coma, survival rates improved significantly by HP over albumin-coated activated charcoal (ACAC) and by *in situ* homologous liver perfusion. In Grade III coma, ACAC HP had no beneficial effect.

Further improvement of survival rates could possibly be realized by an increase of the frequency of HP and/or changing the type of adsorbent (XAD_4, a neutral resin with high affinity for fat-soluble compounds or a combination of charcoal and XAD_4). Therefore, we decided to study the effect of these different types of HP in rats with severe Gal-N-induced hepatitis. As a reference, cross circulation with a healthy inbred donor rat was applied.

MATERIALS AND METHODS

ANIMALS

Male Wistar rats (TNO, Zeist, The Netherlands), weight 300 to 350 gm, were maintained on an unrestricted commercial diet (Hope-Farms). Twenty-four hours before Gal-N administration, rats were starved for 24 hr.

After Gal-N administration, food and liquid (10% glucose) were available *ad libitum*. If spontaneous drinking stopped, 5% glucose was administered intraperitoneally or subcutaneously (5 to 10 ml per 24 hr).

SORBENTS

Activated charcoal (Norit RBXI) coated with cellulose acetate (CA) (4 gm per kg) (Tijssen et al.) (7), Amberlite XAD_4 (Serva, Heidelberg, West Germany) coated with

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either cellulose acetate (6 gm per kg) or polyelectrolyte (PLE) were as described by De Koning et al. (8).

Gal-N HCl (Merck, Darmstadt, West Germany), a solution of 100 mg per ml, was freshly prepared in sterile water and neutralized at pH 7.4 just prior to intraperitoneal injection at a dosage of 1 gm per kg.

Analytical Procedures

SGPT, glucose, and platelet counts were measured according to standard laboratory techniques in clinical chemistry.

A-V Shunt Operation

In order to assess extracorporeal circulation, a shunt [vein-catheter (0.5 × 0.9 mm), B. Braun, Melsungen, West Germany] was applied from the carotid artery to the contralateral jugular vein under pentobarbital anesthesia (50 mg per kg).

HP System

The HP system is shown schematically in Figure 1. Arterial blood is pumped by a small roller pump (Watson-Marlow) to the HP column, through which it flows in an antigravity manner. After passage of the bubble catcher, the blood flows to the venous side of the shunt back into the circulation.

The total system is maintained at 37°C in a water bath and connected by silastic tubing I.D. 0.062, O.D. 0.095 inch. The priming volume of the whole system is about 4 ml, and each HP column contains either 2.7 gm of encapsulated coal or 1.8 gm of encapsulated XAD_4. In the column, the sorbent is covered by two nylon filters and two PMMA support screens.

HP Protocol

Prior to use, the system is recirculated by a heparin-glucose solution (100 ml 5% glucose, 500 units heparin) for about 30 min. Then, the system is primed with 4 ml fresh heparinized (2 units per ml) rat blood (donor rat of the same strain). Except for the priming volume, no additional heparin is used during HP.

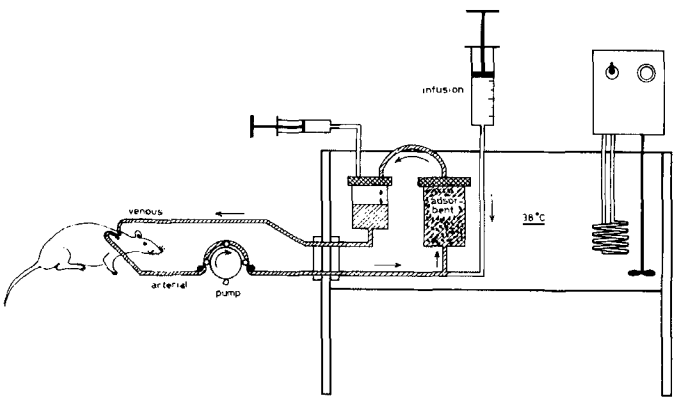

FIG. 1. Schematic drawing of the HP system. Blood is recirculated extracorporeally from the right carotid artery to the left jugular vein by a roller pump and flows through the column in an antigravity manner. The column is filled with approximately 3 gm of the encapsulated sorbent. A bubble catcher is placed between the column and the jugular vein. If neded, heparin can be infused. The whole HP system is thermostated at 37°C in a water bath and connected by silastic tubing I.D. 0.062, O.D. 0.095 inch. During HP, the rat is awake and kept in a restraining age (not drawn).

Under ether anesthesia, the shunt of the "patient rat" is opened and before both sides are connected to the HP system, 1.5 ml of arterial blood is collected for analysis (SGPT), glucose, and platelets). A roller pump provides a flow rate of 2 ml per min.

After finishing HP again, a 1.5-ml blood sample is taken; the rat receives about 3 ml donor blood, and the shunt is restored. During the HP period, the rat is awake and kept in a restraining cage permitting only forward and backward movements over about 5-cm distance. Arterial blood pressure is measured at regular intervals (10 min) by a pressure transducer connected to the arterial line close to the carotid artery.

Time Scale

At time zero, an A-V shunt is implanted during pentobarbital anesthesia and Gal-N is given intraperitoneally. The rat is allowed food and 10% glucose *ad libitum*. Twenty-four hours later, HP is applied as indicated previously. Control animals undergo the same procedure but now HP is applied with a column filled with heparinized fresh rat blood (no adsorbent).

Cross Circulation

During the morning of the day of cross circulation, the donor rat receives an A-V shunt as described previously. During either anesthesia, without heparinization, the patient rat is connected to the donor rat; the last remains anesthesized by pentobarbital (50 mg per kg) and is placed on a scale. A roller pump is connected in between, and the flow rate is set at 2 ml per min. The weight of the donor is checked continuously and speed of in- or outflow is corrected if necessary.

Computer Simulation Program

A Minc-11 (TM), 8-bit mini-computer of the PDP-11 series designed for laboratory support was used connected with a LA 38 matrix printer, producing 30 characters per second. A simulation program describing a two-compartment model, based on linear differential equations as are used in pharmacokinetics (9), was written by one of us (R. P.) (see Figure 2). Assuming relative concentration differences to be the driving force of transport by diffusion between the two compartments, the K_{21} was calculated from the K_{12} by the ratio of the surface interaction of the two postulated compartments [Formula (v)].

The program calculates changing plasma concentration, total body stock, patients' elimination, and HP elimination for the following variable parameters:

P_0 = zero-order production rate in units per hour;
K_{12} = transfer rate from 1st to 2nd compartments;
V_1 = real central volume (liters);
V_2 = real peripheral volume (liters);
Q_1 = partition coefficient of the toxin;
K_{10} = patients own elimination rate;
C = clearing factor of the column (%);
F_2 = flow through the column (liters per min); and

T = perfusing time (hours).

Without HP, the differential equations are:

(i) For the central compartment (V_1)

$$\frac{dX_1}{dt} = P_0 + K_{21}X_2 - K_{12}X_1 - K_{10}X_1$$

$X_{(i)}$ stands for the total stock in the compartment in concern.

(ii) For the peripheral compartment (V_2)

$$\frac{dX_2}{dt} = K_{12}X_1 - K_{21}X_2.$$

(iii) During HP, Formula (i) changes in

$$\frac{dX_1}{dt} = P_0 + K_{21}X_2 - K_{12}X_1 - K_{10}X_1 - K_cX_1.$$

The elimination rate of the column (K_c) is calculated by Formula (iv).

(iv) $$K_c = \frac{C \times F_2}{V_1 \times 100}.$$

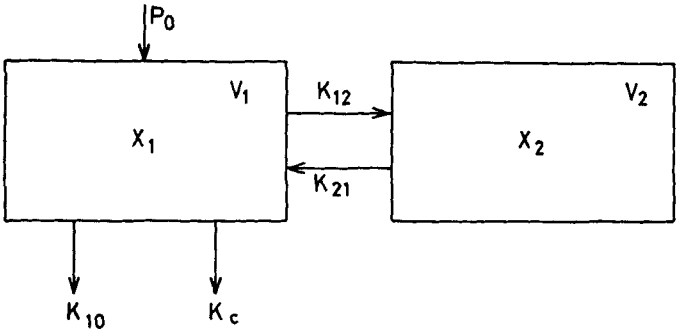

FIG. 2. Two-compartment model. V_1, real central volume; V_2, real peripheral volume; P_0, zero order production rate; K_{12}, transfer rate from 1st to 2nd compartments; K_{21}, transfer rate from 2nd to 1st compartments; K_{10}, patients' own elimination rate; K_c, elimination rate of the column; X_1, X_2, absolute amount of the toxin in V_1 and V_2, respectively.

TABLE 1. SURVIVAL OF HEALTHY CONTROL RATS AFTER HP

Adsorbent	n	Platelet loss[a] (%) (mean ± S.D.)	Survival rate (>14 days)
COAL-CA	5	50 ± 2	100% (5/5)
XAD_4-CA	2	16	100% (2/2)
XAD_4-PLE	4	15 ± 33	100% (4/4)
COAL-CA and XAD_4-PLE	4	21 ± 10	100% (4/4)

[a] Expressed as a percentage of the initial amount.

(v) $$K_{21} = K_{12}\sqrt{3\left(\frac{V_1}{Q_1 \cdot V_2}\right)^2}.$$

RESULTS

HP OF HEALTHY CONTROLS

Table 1 shows the effect of HP through various coated sorbents on survival and platelet loss of healthy rats. The HP "treatment" was initiated 24 hr after provision of the A-V shunt and was continued for 1 hr. It can be seen that blood compatibility was reasonable, and survival was 100% in all cases. Systolic blood pressure varied between 120 to 140 mm Hg, and no severe hypotension was observed.

HP OF GAL-N RATS

Table 2 shows the effect of HP with different adsorbents on survival rate of severe Gal-N hepatitis. No beneficial effect on survival by either treatment could be observed. Most rats died within 2 to 3 days after Gal-N injection. Even repeating HP 3 times over COAL-CA did not improve survival rate.

CROSS CIRCULATION

Cross circulation for 1 or 2 hr initiated 24 hr after Gal-N injection did not improve survival rate as shown in Table 3.

COMPUTER SIMULATION

Figure 3 shows the computer simulation of the effect of HP for 5 hr on the plasma concentration and total body amount of a postulated "toxin". It is evident that if

TABLE 3. SURVIVAL OF SEVERE GAL-N RATS

Treatment[a]	n	SGPT[b] (units/liters) (mean ± S.D.)	Survival rate (>14 days)
Controls	31	1,835 ± 1,350	19% (6/31)
Cross circulation (1 hr)	11	3,200 ± 1,300	0% (0/11)
Cross circulation (2 hr)	6	2,422 ± 808	17% (1/6)

[a] Cross circulation with a healthy donor of the inbred strain was carried out 24 hr after Gal-N injection (1 gm/kg).

[b] Measured 24 hr after Gal-N injection.

TABLE 2. SURVIVAL OF SEVERE GAL-N HEPATITIS

Treatment	n	SGPT[a] (units/liter) (mean ± S.D.)	Platelet loss[b] (%) (mean ± S.D.)	Survival rate (>14 days)
Controls	31	1,835 ± 1,350	ND[c]	19% (6/31)
COAL-CA HP[d]	17	2,045 ± 970	19 ± 12	6% (1/17)
XAD_4-CA HP[d]	6	3,000 ± 2,200	35 ± 17	0% (0/6)
XAD_4-PLE HP[d]	6	2,300 ± 2,040	60 ± 7	0% (0/6)
COAL-CA + XAD_4-PLE HP[d]	7	1,900 ± 1,420	21 ± 10	0% (0/7)
COAL-CA HP (3x)[e]	5	3,625 ± 3,720	ND[c]	0% (0/5)

[a] Measured 24 hr after Gal-N injection.

[b] Expressed as a percentage of the initial amount.

[c] ND, not determined.

[d] HP was carried out for 60 min at time 24 hr after Gal-N injection (1 gm/kg).

[e] HP was carried out for 60 min at time 24, 29, and 48 hr after Gal-N injection (1 gm/kg).

the toxin dissolves equally in water and fat (partition coefficient of 1.0), a HP column with a clearing factor of 100% is very well able to reduce the total body stock of the toxin in a relatively short time.

Figure 4 shows the same simulation but now toxins with a postulated partition coefficient of 50 and 100, respectively, are depicted. Total body stock is hardly influenced.

Table 4 shows the elimination capacity of HP for 5 hr over a 100% effective column in relation to different compartment volumes, different partition coefficients, and different transfer rates of compartment 1 to compartment 2 (K_{12}). Assuming one (arbitrarily chosen) standard body stock value (480 units) at the start of HP, elimination capacity of more than 50% is only obtained at partition coefficients lower than 25 if V_2 is 40 liters and at a partition coefficient lower than 50 if V_2 is 16 liters.

DISCUSSION

The results of HP in healthy controls show that biocompatibility of the procedure is very acceptable. This is in agreement with the *in vitro* results of blood compatibility as reported by De Koning (10). In Gal-N hepatitis rats, no fatal effect of HP could be observed on blood compatibility and arterial blood pressure under our experimental conditions. Postmortem microscopic inspection of liver, heart, lungs, and kidney showed no other abnormalities than those contributable to severe Gal-N hepatitis as described by Decker and Keppler (11).

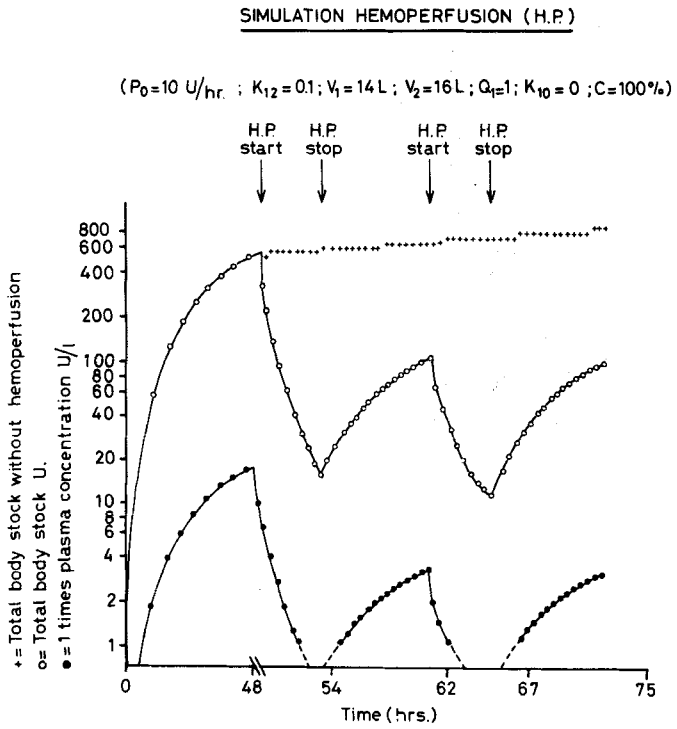

FIG. 3. Computer simulation of HP effect on total body stock and plasma concentration of the "toxin". At time 0, zero order production rate starts at 10 units per hr. K_{12}, 0.1. Elimination rate of the patient is 0. Central volume (V_1) is 14 liters. Peripheral volume (V_2) is 16 liters. Partition coefficent (Q_1) is 1. Flow through the column: 0.5 liter per min. Clearing factor of the column (C) is 100%. Perfusion time is 5 hr.

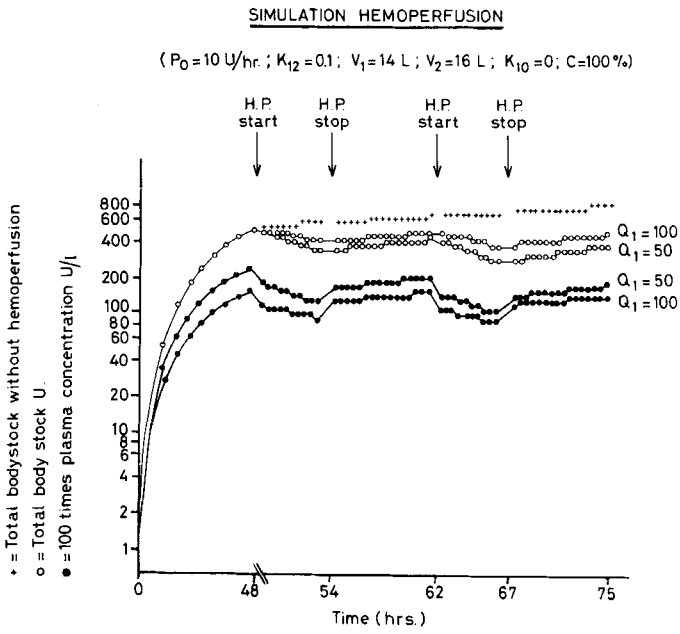

FIG. 4. Computer simulation of HP effect on total body stock and plasma concentration of the "toxin". At time 0, zero order production rate starts at 10 units per hr. K_{12} is 0.1. Elimination rate of the patient is 0. Central volume (V_1) is 14 liters. Peripheral volume (V_2) is 16 liters. Two different partition coefficients (Q_1) are shown: 50 and 100. Flow through the column is 0.5 liter per min. Clearing factor of the column (C) is 100%. Perfusion time is 5 hr.

TABLE 4. COMPUTER-SIMULATED ELIMINATION CAPACITY OF HP

K_{12}	Column elimination capacity[a] (%)				
	$Q_1 = 1$	$Q_1 = 10$	$Q_1 = 25$	$Q_1 = 50$	$Q_1 = 100$
	$V_1 = 14$ and $V_2 = 16$ liters				
0.01	90	47	33	25	20
0.10	97	74	55	40	29
0.40	98	79	60	45	32
	$V_1 = 14$ and $V_2 = 40$ liters				
0.01	75	33	23	18	15
0.10	93	55	36	26	18
0.40	95	60	41	29	20

[a] Defined as per cent of the postulated total body stock of the toxin at 53 hr if no HP had been applied. P_0, 10 units/hr; HP is started after 48 hr; K_{10}, 0; C, 100%; F_2, 0.5 (liter/min); T, 5 hr; Q_1, partition coefficient of the toxin.

Our results show no beneficial effect on survival rate from severe Gal-N hepatitis neither by HP over encapsulated sorbents (coal, XAD_4, or a combination of both) neither by cross circulation. This is in agreement with the results of Chang et al. (6, 12–14) as far as Grade III coma rats are concerned, although they use a slightly different methodology (Gal-N dosage, 1.1 gm per kg and starting HP at a time of 48 hr). Since under our experimental conditions almost all animals reach coma Grade III at about 48 hr and do not survive a period of more than 50 to 55 hr, our rats cannot be compared with Chang's favorable results in Grade II coma rats. Furthermore, since it is a common experience that big dif-

ferences in survival rate can exist between batches of animals with the same high dose of Gal-N (14), the conclusion seems justifiable that rats in coma Grade II at 48 hr after Gal-N injection belong to a "privileged" category.

If HP were able to improve survival rate of Grade III coma rats, starting this treatment at 24 hr instead of at 48 hr after Gal-N injection would increase rather than decrease survival rate, extrapolating the recent results of Gimson et al. in human fulminant hepatic failure (15). On theoretical grounds, however, it seems very unlikely that HP or cross circulation for 1 or 2 hr a day will be sufficient to replace effectively the failing detoxification function of acute hepatic failure when accumulation of toxic factors most probably occurs continuously. In favor of this idea is the reported evidence that long-term treatment (e.g., hemofiltration) has improved consciousness in patients (16).

Our results obtained by computer simulation show clearly that HP is only effective in removing toxic substances with a small distribution volume (low partition coefficient) and that the outcome is hardly influenced by rather large changes in transfer rates (K_{12} and K_{21}) between the two compartments (Table 4). This is in agreement with the computer simulation of hemoperfusion by Berk (17) applied to unconjugated bilirubin. His data show that even if 50% of the total body stock of the presumed "toxin" (unconjugated bilirubin) is present in the central volumes (plasma), a 3-day period of 4-hr HP each day is needed before normal plasma values are obtained. These results are quite comparable with our calculation of a "toxin" with a partition coefficient of about 1.0 (Figure 3). Although strong binding of the presumed "toxin" to albumin hampers significantly the efficacy of HP treatment (18, 19), a high partition coefficient of the "toxin" is a far more restrictive factor for success of this type of treatment.

Since hepatic biotransformation concerns especially metabolic waste products with low polarity, high-protein binding, and/or high-fat solubility, it seems very likely that potential comagenic factors in HE will have a high partition coefficient. Considering this, we are not surprised that intermittently applied HP for a relatively short time is not a successful treatment of severe hepatic failure.

Therefore, we suggest that an important feature of artificial support of the failing hepatic detoxification function should be efficiency in removing substsances with a high partition coefficient. Such an artificial liver support will certainly be more successful if it can be applied in an early phase of acute hepatic failure and if it can be given continuously.

Peritoneal dialysis has more or less these characteristics depending on the composition of the dialysate. Experiments are in progress to test this hypothesis.

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